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Anti-bacterial activity of *Cannabis sativa* Linn. leaf extracts against different pathogenic bacterial strains

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Several compounds are present in Plants which are used for therapeutic purpose. The medicinal plant can use for the curing of different diseases because of these biologically active compounds. The aim of this study was conducted to determine the antibacterial activity of Cannabis sativa plant against different bacterial strains such as E. coli, B.subtilis, Shigella, Staphylococcus aureus, Pseudomonas aeruginosa and Klebsila. Five different crude extracts were prepared from the leaves of Cannabis sativa. The n-butanol, Ethyl acetate, n-hexane and methanol fractions crude and DMSO extracts were evaluated for their antibacterial potential by disc diffusion method.

Keywords: E. coli, B. subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsila

INTRODUCTION

Cellular activities of plants work under favorable environments alike growth, development, respiration etc. Any harsh condition

as a consequence of biotic and abiotic hassles interrupts these cellular activities leading towards the repression of immune system. The annihilation of immune system leads to infectious diseases and their harmful consequences. Antibiotics and chemotherapeutic agents have been administrated in order to control the progression of pathogens and many studies have been carried out to study such effects. But generally, bacteria having ability to show resistance to drugs, as a result new bacterial strains which are multi resistance. This may be a high threat and increases mortality rate (Nascimento et al. 2000). In addition, antibiotics also associated some adverse effects on host i.e. hypersensitivity, immunosuppression and allergic reactions (Shah et al., 2011; Hazrat et al., 2020). Therefore, these synthetic drugs should be replaced with natural sources of treatments. However, in this regard medicinal plants have been used conventionally by both the forks of primitive and modern era for handling various diseases (Ali et al., 2020; Bakht et al. 2013a). According to WHO report, traditional medicines are used by most of the world's population more than 80percent for health care. Moreover, medicinal plants have more advantages over synthetic drugs in term of their effectiveness, cost and effectiveness (Bhatia et al., 2006; Bakht et al., 2011b).

C. sativa like other medicinal plants contain active ingredients which are used for therapeutic purpose (Kuddus et al. 2013). In cannabis plant about 400 compounds and more than 60 cannabinoids have been identified (Turner et al., 1998). In leaf of *C. sativa*, presence of different phytochemicals like tannins, phenols, flavonoids, terpenes, alkaloid, cardiac glucosides and steroids, resins volatile oil has also been identified (Sofowora et al. 1982; Wagnar et al. 1983). *C. sativa* has anti-inflammatory, sedative and analgesic activity (Janet et al. 1999). The clinical aspect of *Cannabis* is that it is also used as medicine gynecological clinicians. Cannabis extract are also used in extensive range of disorder in women like menopausal symptoms, dysuria and hyperemesis gravidarum (Russo, 2002). Plants extracts are also used by humans as seed oils and stalk and both of these are also used as a fuel (Clarke, 2002). Another major advantage of cannabis is that it was used in 20th century B.C in Egypt for treating sore eyes, bhang used as anti- phlegmatic and as anesthetic. Various conditions includes, rabies, cholera, rheumatism and tetanus, are cured with cannabis. Many human diseases like allergies, cuts, wounds, inflammation, burns, small pox and sexually transmitted diseases are cured by using *C. sativa* (Dilara and Nath, 2000). *C. sativa* potential has been accepted world widely have the potential through which several diseases can be cured like from cancer to headache and various nervous disorders. The main objective of this study was to use the leaf extract of C. sativa plant against disease causing bacteria.

MATERIALS AND METHODS

Plant materials

Sample collection:

The health mature and disease free leaves were collected from Peshawar Pakistan.

Preparation of crude extract

For extracts preparation, First leaves were washed properly with distal water and were placed in room temperature to get dried. Then leaves were grinded in a grinder to make fine powder. The grinded powder was placed in the flask and then weighted. The fine powder about 200g was placed in a large container. The fine powder was completely dipped in a 90% methanol to yield the crude extract.

Fractionation of crud extract

In order to make different fractions of the extract, five different solvents were used in fractionation. Firstly, 30mg dried methanol extract was dissolved in 300ml distilled water. Then the solution was transferred to separate funnel and calculated amount of n-hexane i.e 300ml was added to it. The solution was vigorously shaked, and allowed to make two phases. The upper phase contained n-hexane while the lower phase contained water. Both solvents being immiscible in each others were collected.

Culture media and its preparation

Nutrient agar media (HiMedia Laboratories Pvt., Ltd.) was used for the culture and growth of microorganisms while nutrient broth was used for the incubation and standardization respectively. The calculated amount of nutrient agar and nutrient broth were prepared accordingly and then poured media in a conical flask. Before pouring all the required equipment were properly sterilized in autoclave before pouring media in petri plates. The cultured microorganisms in nutrient broth which were kept in shaking incubators at 28ºC for

24 hours were streaked on plates by sterilized loop. After streaking, these plates were allowed for a few minutes and then disc (made from Whatman filter paper) were placed at three different positions that were labeled for different concentrations. Finally, plant extract of different solvent fractions were poured on these disc at 6µl, 12µl, and 18µl concentrations. These plates were incubated at 37ºC for overnight.

Microbes Used

The in-vitro activity of C. sativa extracts was screened against different bacterial strains like E. coli, B.subtilis, Shigella, Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumonia.

Disc diffusion susceptibility assay

The antibacterial activity of different solvent extracts of C. sativa was tested by disc diffusion method according to Bakht et al. (2014). Filter paper (Whatman No. 1) discs 6mm in diameter were placed on nutrient agar media plates with help of sterile forceps and then plant extracts were applied in concentration of 1, 2 and 3 mg disc-1 in 6, 12 and 18µl volume on the discs. Antibiotics for bacteria = Ciprofloxacin 50 µg per 6 µl and for Candida albicans = flucanozole 50 µg per 6 µl used as positive control. DMSO (6 µl disc-1) as negative control was also applied on the discs. Then these inoculated plates were incubated at 37ºC for overnight. After 24 hrs, zone of inhibition around each discs were calculated in mm.

RESULTS AND DISCUSSION

In the current study, the leaf extract of c. sativa were used against 4 gram negative and 2 gram positive bacteria. The activity of the extracts was based on zone of inhibition. The extract of different solvent fractions was poured on this disc at 6µl, 12µl, and 18µl concentrations. The results of all concentration were shown in table 1, 2, and 3 and figure 1, 2, 3 respectively. The antimicrobial activity of the extracts at 18µl was maximum as compared with the other concentrations. The zone of inhibition was measured in mm. the p value in all the concentration was less than 0.001 as compared to control.

C. sativa like other medicinal plants contain active ingredients which are used for therapeutic purpose (Kuddus et al., 2013; Shiheng et al., 2017). In cannabis plant about 400 compounds and more than 60 cannabinoids have been identified (Turner et al., 1998). C. sativa has anti-inflammatory, sedative and analgesic activity (Janet et al., 1999). In the current study, the leaf extract of cabanas sativa were used against pathogenic bacteria. The 2 bacteria were gram positive and 4 were gram negative. The activity of the leaf extract was based on the zone of inhibition. The activity of the extracts wills positive if zone of inhibition appeared other vise no zones no activity. The outcomes of the study were summarized in result as shown in table no 1, 2 and 3. The solvent extracts in different concentration were used against the bacteria. In all the concentration, the extract showed activity but the range of these extracts was different in different concentration. The maximum ZOI 18.16±0.23 mm were shown by n-haxane extracts against E.coli as shown in Fig 4.

Table 1: Antimicrobial activity of leaf extract of C.Sativa at 6µl P value<0.001

S.No	Bacterial strains	Butanol Mean+SD	n-haxane Mean+SD	Ethyl acetate Mean+SD	Methanol Mean+SD	DMSO (Control)
1	E. colia (-ve)	7.16±0.23mm	6.51±0.40 mm	6.73±0.20 mm	9.96± 0.54 mm	0
2	B.subtilis (+)	11.26±0.37 mm	10.43±0.41 mm	11.57±0.40 mm	8.13±0.12 mm	0
3	Shigella(-ve)	6.26±0.37 mm	10.16±0.23 mm	9.88±0.15 mm	13.20±0.64 mm	0
4	Klebsiella pneumonia (-ve)	7.11±0.23 mm	6.66±0.23 mm	7.13±0.18 mm	10.68±0.22 mm	0
5	S. aureus(+)	4.62±0.37 mm	6.75±0.74 mm	7.56±0.24 mm	7.84±0.21 mm	0
6	Pseudomonas aeruginosa(-ve)	1.80±0.35 mm	2.37±0.26 mm	1.13±0.18 mm	4.61±0.28 mm	0

Table 2: Antimicrobial activity of leaf extract of C.Sativa at 12µl P value<0.001

S.No	Bacterial strains	Butanol Mean+SD	n-haxane Mean+SD	Ethyl acetate Mean+SD	Methanol Mean+SD	DMSO (Control)
1	E. colia (-ve)	11.15±0.14 mm	9.37±0.21 mm	7.06±0.32 mm	10.68±0.15 mm	0
2	B.subtilis (+)	11.93±0.09 mm	13.43±0.61 mm	12.83±0.23 mm	10.46±0.38 mm	0
3	Shigella(-ve)	6.26±0.37 mm	10.16±0.23 mm	9.88±0.15 mm	13.20±0.64 mm	0
4	Klebsiella pneumonia (-ve)	9.16±0.23 mm	12.4±0.32 mm	8.73±0.24 mm	10.95±0.36 mm	0
5	S. aureus(+)	6.62±0.37 mm	6.09±0.30 mm	8.89±0.32 mm	8.29±0.49 mm	0
6	Pseudomonas aeruginosa(-ve)	4.46±0.38 mm	5.03±0.36 mm	5.76±0.20 mm	9.40±0.36 mm	0

Table 3: Antimicrobial activity of leaf extract of *C.Sativa* at 18µl P value<0.001

S.No	Bacterial strains	Butanol Mean+SD	n-haxane Mean+SD	Ethyl acetate Mean+SD	Methanol Mean+SD	DMSO (Control)
1	E. colia (-ve)	13.06±0.24 mm	18.16±0.23 mm	14.83±0.62 mm	13.83±0.12 mm	0
2	B.subtilis (+)	12.21±0.32 mm	19.06±0.09 mm	15.21±0.14 mm	12.56±0.16 mm	0
3	Shigella(-ve)	11.95±0.14 mm	18.86±0.18 mm	15.8±30.23 mm	12.26±0.37 mm	0
4	Klebsiella pneumonia (-ve)	14.14±0.18 mm	14.03±0.20 mm	12.13±0.18 mm	11.26±0.20 mm	0
5	S. aureus(+)	13.73±0.24 mm	12.28±0.16 mm	13.2±0.16 mm	10.23±0.28 mm	0
6	Pseudomonas aeruginosa(-ve)	11.16±0.12 mm	12.43±0.61 mm	10.13±0.18 mm	11.83±0.23 mm	0

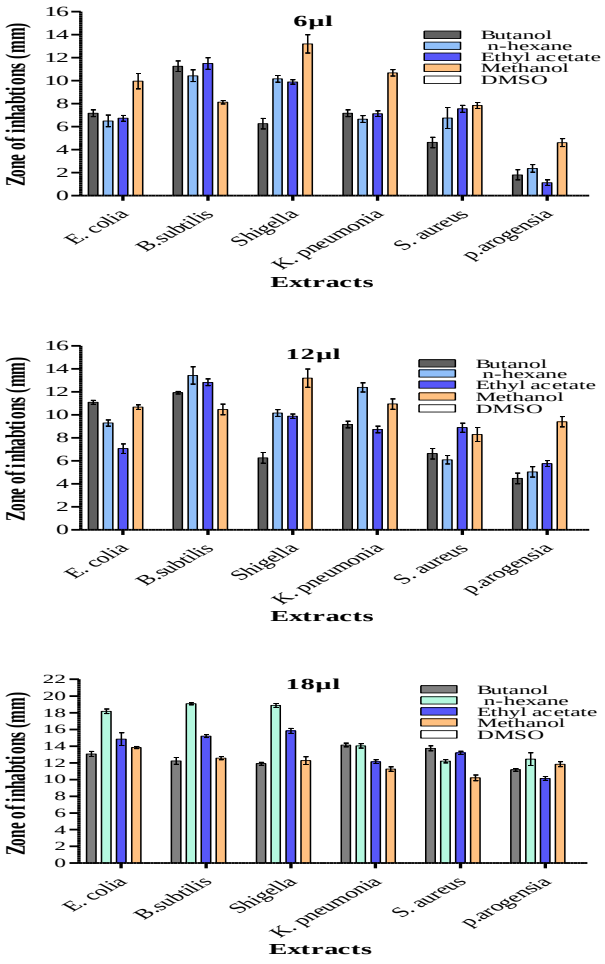

Figure 1: Different extracts activity of *C. Sativa*

Our results are in agreement with (Naveed et al. 2014) who reported that leaf extract of *C. sativa* showed well defined antibacterial activity against Staphylococcus aureus (24.1mm) and E .coli (22.2mm). For B. subtilus and Shigella the same extract showed wider zone of inhibation 19.06±0.09 mm and 18.86±0.18 mm respectively. The ZOI 14.14±0.18 mm and 13.73±0.24 mm were observed in butanol extracts against Klebsiella pneumonia and S. aureus respectively, while for Pseudomonas aeruginosa the ZOI 12.43±0.61 mm were shown by n-haxane extract. Similarly (Esra et al. 2012; Tabinda et al., 2020; Romman et al., 2015) also investigated Can-nabis sativa extract as a antimicrobial agent against Bacillus subtilis, Staphylococcus aureus Escherichia coli and Pseudomonas aeruginosa, the ZOI were (21 mm, 28 mm, 15 mm and 16 mm respectively. The ZOI for the mentioned bacterial strain with were 15mm, 21mm, 28mm and 16mm. (Monika et al. 2014; Bilqees et al., 2020; Romman et al., 2020) screened the activity of C. sativa against different pathogen. The highest zone 22mm and 19mm were observed for Escherichia coli, and Staphylococcus aureus. Similarly Verma et al. (2014) also investigated C. sativa against Staphylococcus aureus which showed moderate to good activity.

CONCLUSION

The extract showed activity against all the bacteria but the rate of susceptibility was different in different microorganisms. Numerous microbial diseases can be cure through Cannabis sativa which have antimicrobial elements. From the results it is concluded that Cannabis sativa the extracts showed against all the selective bacteria. Although the extract of the selective plant showed activity against these bacteria but it needs to investigated the secondary metabolites. In the marked the antimicrobial activity are present but they are very costly, low effective and very toxic with passage of time. For the bacterial infection, its need to obtained medicine from plant. The origin for the bacterial therapy should be plant therapy. So it need to discovered antimicrobial agent, which are very effective and fulfill the present demand..

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

MA designed and performleed the experiments and also wrote the manuscript. MA and MR performed animal treatments and data analysis. SU designed experiments and RP, MS, SB, AAKK, MK, FH, SJ, SSSH, RB, WK, BA, AH, NU, NM, ZU, IUH, HAJ, SU, MI reviewed the manuscript. All authors read and approved the final version.

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